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Author(s)	Saito, Yasunori; Mukai, Hideo; Watanabe, Hiroshi
Citation	PUBLICATIONS OF THE SETO MARINE BIOLOGICAL LABORATORY (1981), 26(4-6): 357-368
Issue Date	1981-09-30
URL	http://hdl.handle.net/2433/176032
Right	
Type	Departmental Bulletin Paper
Textversion	publisher

STUDIES ON JAPANESE COMPOUND STYELID ASCIDIANS II. A NEW SPECIES OF THE GENUS *BOTRYLLOIDES* AND REDESCRIPTION OF *B. VIOLACEUS* OKA[1)]

YASUNORI SAITO

Shimoda Marine Research Center, University of Tsukuba, Shimoda, 415 Japan

HIDEO MUKAI

Department of Biology, Faculty of Education, Gunma University, Maebashi, 317 Japan

and

HIROSHI WATANABE

Shimoda Marine Research Center, University of Tsukuba, Shimoda, 415 Japan

With Text-figures 1–5

Since 1959, a species of the genus *Botrylloides,* commonly found on the rocky shore in the vicinity of the Shimoda Marine Research Center, the University of Tsukuba and frequently used as the material for experiments at the center, has been identified by the present authors as *Botrylloides violaceus* Oka. The colonies of this botryllid release larvae, each furnished on the trunk with 8 ampullae that are elongated after attachment. Then in 1978, some colonies of another form of *Botrylloides* were collected near the Shimoda Marine Floating Aquarium. These colonies released larvae larger and furnished with about 30 ampullae, though the feature of colony and morphology of zooids were almost the same as in the above-mentioned species releasing larvae with 8 ampullae. Since then, respective morphological characters and the life history have been compared with each other in detail between these two forms. Through these careful observations, it has become clear that the two forms of *Botrylloides* mentioned above are quite distinct from each other. Thus the authors are now facing a taxonomic problem to determine by which of these two forms *Botrylloides violaceus* should be represented. *B. violaceus* was established by Oka (1927) with a very short description as noted by Tokioka (1953, pp. 242–243), without information on its type locality. Very unfortunately, no types of this species were designated by Oka. At the present level of knowledge attained by closer observations of zooidal morphology and life history in the two forms of *Botrylloides* under consideration, Oka's description and also Tokioka's made later on many colonies from different localities are insufficient to separate these two forms from each other. If the locality were limited to a small region, the probable identification

1) Contributions from the Shimoda Marine Research Center, No. 385.

Publ. Seto Mar. Biol. Lab., XXVI(4/6), 357-368, 1981. (Article 20)

might have been done more easily. Actually, however, colonies were gathered by these authors from so wide range. Therefore, it is natural to consider that the two forms under consideration, and probably some other botryllids referred to in other papers of this series, are included in the descriptions given by those two authors. The simplest solution of the present problem is to put *B. violaceus* aside and establish two new species for these two forms. However, in a hope to conserve the name of *violaceus* given by Oka who was a pioneer in this country of the taxonomic study of ascidians, the present authors have decided after a long time of hesitation to designate the form releasing larvae furnished with 30 ampullae to *B. violaceus* as explained later in more detail, and to propose a new species for the form releasing larvae with 8 ampullae. In the present paper, both the new species and *B. violaceus* are described or redescribed respectively and their life histories are given in detail to demonstrate their significance in the taxonomy of compound styelids.

Before going further, the authors want to present their sincere thanks to Dr. Takasi Tokioka, Professor Emeritus of Kyoto University, and Mr. Teruaki Nishikawa of the College of General Education, Nagoya University, for their suggestions and advices in taxonomy and to the former for his kindness in reading the manuscript critically. They are also deeply indebted to the members of the Shimoda Marine Reseach Center for their kind assistance.

Botrylloides simodensis Saito and Watanabe n. sp.

(Figs. 1–2)

The present species is found in the lower intertidal zone, commonly on the surface of stones, rocks or sea weeds in tide pools or in rocky clefts near the Research Center, and also is known from Misaki, Kanagawa Prefecture; Shirahama, Wakayama Prefecture; and Mukaishima, Hiroshima Prefecture. The holotype and paratype colonies are deposited at the Research Center; type locality, Shimoda.

Description: Colonies are usually encrusting and sometimes attain a large size more than 10 centimeters across; usually 2.0–3.0 mm in thickness. The colony surface is flat and free from any foreign matter. The test is soft gelatinous and almost transparent. When alive, they are colored yellowish brown, dark brown or orange. In addition, some of red, black, green, purple or white pigment cells are frequently deposited around the branchial siphon and on the atrial languet of respective zooids. Thus, usually different color patterns are represented on the colony surface. The periphery of the colony is fringed by sausage-shaped vascular ampullae about 1500 μ long and 300 μ wide.

Zooids (Fig. 1a) are arranged in ladder systems with several common cloacal apertures, and are always connected one another by a common vascular system. They are 2.0–2.5 mm in length and situated more or less vertically standing, though obliquely in the periphery. Branchial tentacles consist of 4 larger and 4 smaller ones, regularly alternating and additionally 1–2 minute ones in some interspaces.

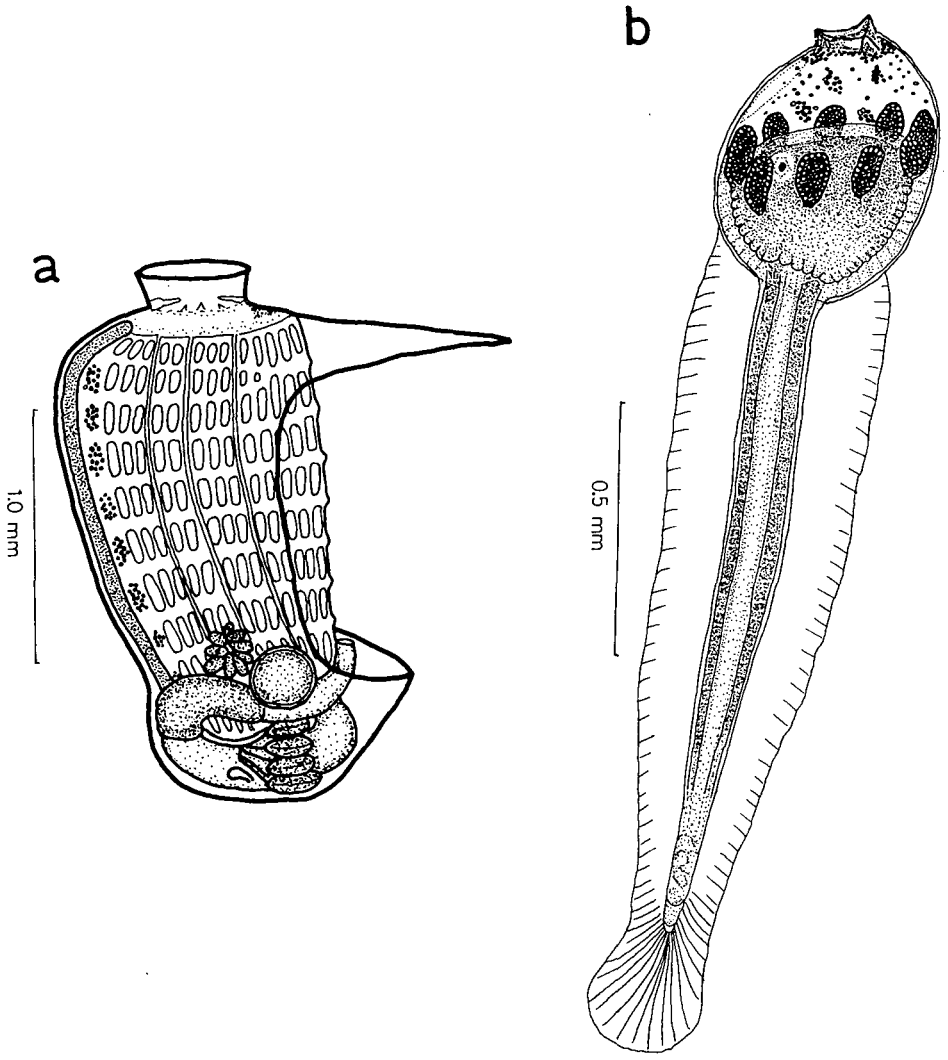


Fig. 1. *Botrylloides simodensis* n. sp.. a, a zooid, from left side. b, a larva, from left side.

There are 11 rows of stigmata on each side; the second row never reaches the dorso-median line. Around the middle of the branchial sac, stigmata are arranged between the 3 inner longitudinal bars as follows: dorsal lamina 5·2–3·2–3·3–4 endostyle. Many blood cells are deposited along each side of the endostyle in the range from the second to the ninth stigmatal row. The anterior edge of the intestinal loop attains anteriorly the level of the 9th transverse vessel and the anus opens at the level of the 9th stigmatal row. The most part of the stomach is exposed posterior to the rear end of the branchial sac. The stomach is orange in color in fresh specimens and is furnished with 9 longitudinal plications and a small pyloric coecum.

The testis is situated along the anterior edge of the circum-intestinal gland region on the left side and at the level of the tenth transverse vessel on the right side,

and anterior to the ovary. It consists of several lobes forming a rosette, and is colored grayish. Usually a single egg is matured on each side; it is yellowish orange in color and approximately 250 μ in diameter. The incubatory pouch is a sac-like outgrowth of the body formed near the ovary and the development of the fertilized egg takes place within it, and the embryo in the pouch grows to about 300 μ in diameter.

The larva (Fig. 1b) is about 1.7–2.0 mm in total length and yellowish orange in color when alive. The trunk is about 500 μ long, oval in outline and includes a single statolith as typical to botryllids; three attachment processes are arranged in a triangle and 8 ampullae are arranged to form a circular ampullar band surrounding the middle part of the trunk.

Life history: The asexual peribranchial budding can be observed all through the year; usually a single bud is produced on each side of the body. The sexual reproduction is limited to the warm water season from July to September, with a peak in August. The egg attains to its maximal size in the ovary already in the stage of bud. The egg is ovulated into the incubatory pouch soon after the alternation of generation and there develops into the tadpole larva in 4 or 5 days. Larvae are released from the colony in the morning from 10:00 to 12:00, before their parent zooids degenerate. Two to five hours after liberation, larvae become attached to the substratum by extension of 8 ampullae, and the metamorphosis is started (Fig. 2). The first bud always appears only on the right side of the attached individual before it becomes functional.

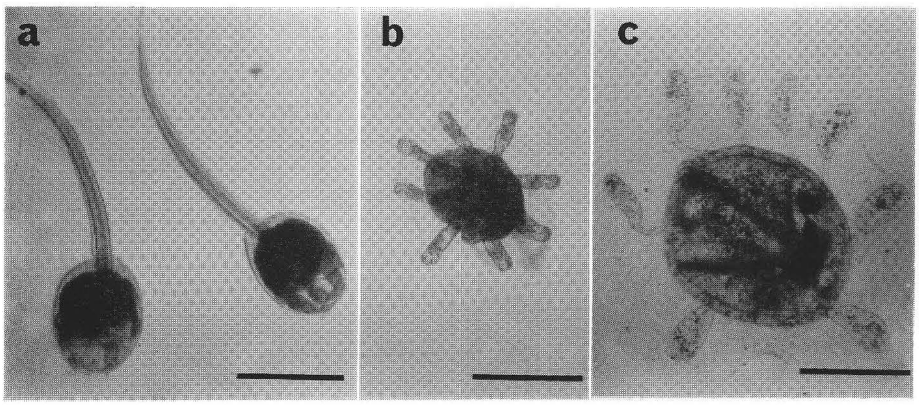


Fig. 2. *Botrylloides simodensis* n. sp.. a, swimming larvae. b, an oozooid in metamorphosis, 4 hours after larval attachment; ventral view. c, the same, 24 hours after attachment; ventral view. Each scale bar indicates 500 μ.

Botrylloides violaceus Oka nom. cons.

(Figs. 3–5)

Colonies of the present species, redescribed here, are found around Shimoda limitedly near the Shimoda Marine Floating Aquarium in still water of 20–150 cm

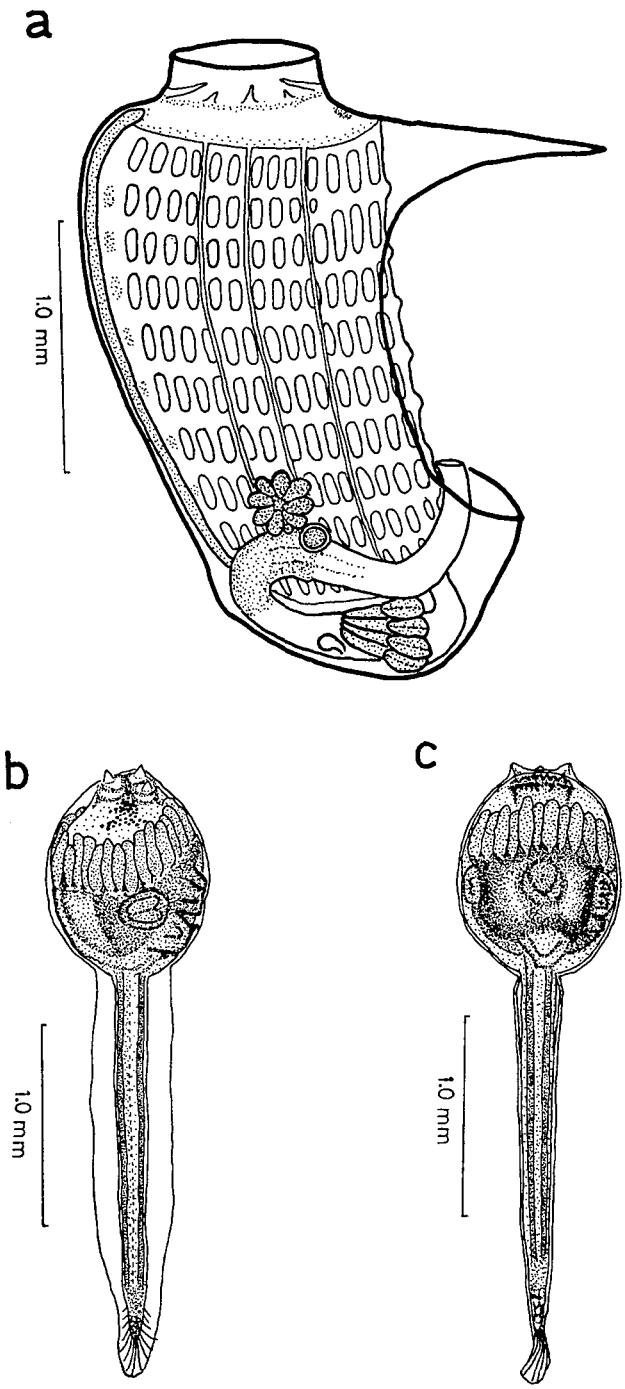


Fig. 3. *Botrylloides violaceus* Oka. a, a zooid, from left side. b, a larva, from left side. c, the same, from dorsal side.

deep, usually covering the surface of algae and shells, such as *Mytilus*. On the other hand, this species occurs commonly around Akkeshi on the southeastern coast of Hokkaido Island and Asamushi on the coast of Mutsu Bay, Aomori Prefecture. The specimens, on which the present redescription is made, are all deposited as the neotypes at the Research Center.

Description: Colonies are generally encrusting and sometimes attaining a size about 10 centimeters across; usually 2.0–3.0 mm in thickness. The colony surface is flat and free from any foreign matter. The test is soft gelatinous, almost transparent and colorless. The colonies may look dark brown, yellow or whitish when alive. The periphery of the colony is fringed by sausage-shaped vascular ampullae about 500 μ long and 200 μ wide.

Zooids (Fig. 3a) are arranged in ladder systems with several common cloacal apertures, though the systems become often obscured by crowding of zooids, and are always connected one another by a common vascular system. They are 2.5–3.0 mm in length and more or less vertically standing, though laid obliquely in the periphery. Branchial tentacles consist of 4 larger and 4 smaller ones alternating regularly and additionally 2–3 minute ones in some interspaces. There are 10–11 rows of stigmata on each side; the second row never reaches the dorso-median line. Around the middle of the branchial sac, stigmata are arranged between the 3 inner longitudinal bars as follows: dorsal lamina 4·3–4·2·4–5 endostyle. The anterior edge of the intestinal loop attains to the level of the 9th stigmatal row and the anus opens at the level of the 7th transverse vessel. The stomach is wholly exposed posterior to the rear end of the branchial sac. It is furnished with 9 longitudinal plications and a very small pyloric coecum.

The testis is situated along the anterior edge of the circum-intestinal gland region on the left side and at the level of the 10th transverse vessel on the right side, and anterior to the ovary. It consists of several lobes forming a rosette. Usually a single egg is matured on each side, sometimes on the right side only. The egg is transparent, light yellow in color and less than 100 μ in diameter. The incubatory pouch is a sac-like outgrowth of the body formed near the ovary and the pouch including an embryo remains in the test after the degeneration of the parent zooid till the tadpole hatches out. The embryo in the pouch grows to a size of 1.0–1.2 mm in diameter.

The larva (Fig. 3b, c) is about 3.0 mm in total length and dark brown or yellowish orange in color when alive. The trunk is about 1.0 mm long and oval in outline; three attachment processes are arranged in a triangle and 24–34 ampullae are arranged to form a circular ampullar band surrounding the anterior part of the trunk. There are three buds around the lateral sides of the trunk, one on the left and two on the right side. Heartbeating is recognizable already in the stage of swimming larva.

Life history: The asexual peribranchial budding can be observed all through the year. Each zooid usually produces two buds on each side of the body, though the number of buds is seemingly diminished in the sexual breeding season from May to August, with a peak in July. The ovarian egg in the bud will be ovulated soon

after the bud is grown to be an adult zooid taking the place of its degenerated parent zooid. The egg at this time is about 90 μ in diameter and contains little yolk. The ovulated egg goes into the incubatory pouch and there the embryogenesis is started. About 5 days after the ovulation, zooids bearing embryos in pouches are degenerated, leaving the pouches in the test but the connection between the pouches and the colonial vascular system still remaining. The size of embryo at this time is still as small as 100 μ in diameter, but in about 5 weeks after the ovulation the embryo grows up in the pouch to the larva of a size 1.0–1.2 mm in diameter, while the alternation of generation of zooids takes place 6 or 7 times. Thus, embryos of 6 or 7 successive stages may be observable in the pouches found in the same, single colony at the peak of sexual reproduction.

Fully developed larvae are released from the incubatory pouch and swim out from the common cloacal apertures of the colony. However, hatching is not synchronized, larvae are released from the parent colony all day. Four to 10 hours after liberation, the larvae become attached to the substratum by extension of 24–34 ampullae. The number of first ampullae is variable even in the larvae derived

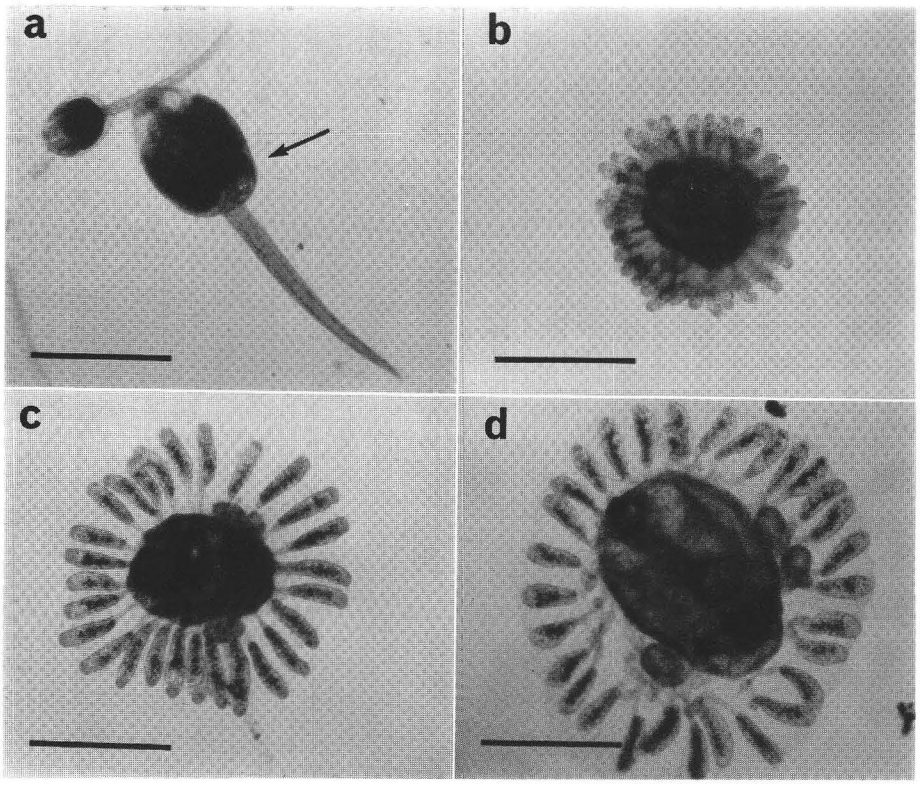


Fig. 4. *Botrylloides violaceus* Oka. Stages in the metamorphosis from larva to oozooid. a, a swimming larva (pointed by arrow). Smaller one of *B. simodensis* is shown for comparison. b, an oozooid in metamorphosis, 4 hours after larval attachment; dorsal view. c, the same, 8 hours after attachment; dorsal view. d, the same, 24 hours after attachment; dorsal view. Each scale bar indicates 1.0 mm.

from the same colony. The metamorphosis (Fig. 4) goes so fast that both the branchial and atrial siphons are opened about 6 hours after the attachment.

Summary and Considerations

Differences between *Botrylloides simodensis* n. sp. and *B. violaceus* Oka, that may be confirmed in preserved specimens, are tabulated below.

	B. simodensis	*B. violaceus*
peripheral vascular ampullae	1500 μ long $\times 300\ \mu$ wide	500 μ long $\times 200\ \mu$ wide
zooids	2.0–2.5 mm long	2.5–3.0 mm long
stigmatal rows	11	10–11
anus opening at the level of	9th stigmatal row	7th transverse vessel
egg diameter	250 μ	$<100\ \mu$
embryo diameter	300 μ	1–1.2 mm
larva	1.7–2.0 mm long	3.0 mm long
larval trunk	500 μ long	1.0 mm long
number of ampullae	8	24–34

From this table, it is evident that the separation of the two species from each other is extremely difficult when the preserved colonies are not in the sexual breeding phase. On the contrary, the distinction between the two species can be made much more easily on living colonies. Even in the live colonies, not in the sexual breeding season, the two species may be separable from each other by coloration to some extent. The coloration is rather uniform in *B. violaceus*, but rich of variable patterns in *B. simodensis*. Especially, when they are in the sexual breeding season, many prominent differences are manifested between the two species in their life history, inclusive of embryogenesis, as seen below.

	B. simodensis	*B. violaceus*
production of larva	ovoviviparous as seen generally in the genus	viviparous, seen rather rarely in ascidians
yolk amount	large	little
embryo size in incubatory pouch	hardly increasing	grow larger
development to larva	in 4–5 days	more than a month
release of larvae	shortly before degeneration of parent zooid	after 6–7 times of alternation of generation of zooids

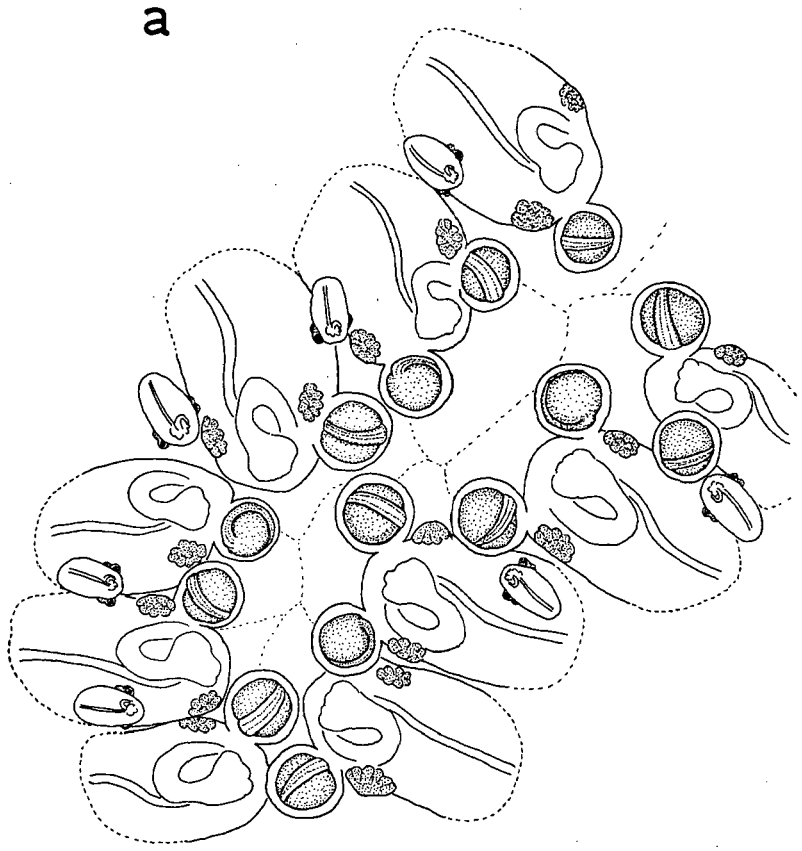
These differences are summarized in the feature of colonies containing incubatory

pouches as follows. In *B. simodensis,* the incubatory pouches are always found connected with their parent zooids and all embryos within the pouch are in the same developmental stage in the same colony (Fig. 5a). In *B. violaceus,* however, the incubatory pouches are left in the test keeping a connection only to the common vascular system but not to any zooids, and contain within them respectively the embryos of several different developmental stages in the same colony (Fig. 5b).

The two species are seemingly differentiated also in their geographical distribution. *B. simodensis* is clearly distributed much more richly in the warm water region, though it occurs poorly in Akkeshi and Asamushi. On the contrary, *B. violaceus* is seemingly a form mainly distributed in the boreal waters, occurring commonly in Akkeshi and Asamushi, but only rarely in Shimoda.

As the sexual breeding is limited to the summer season in both species, while the big low-tides when the habitats of these species are exposed, occur in the spring season in this country, it is natural that most preserved colonies treated with in previous taxonomic studies are devoid of incubatory pouches containing fully developed embryos, or no released tadpoles are remaining inside those colonies. Thus, inevitably *B. violaceus* described so far has been a polyphyletic taxon as noted already by specialists in this country. In order to analyse this species, the number of stigmatal rows and that of plications on the stomach surface have been recorded repeatedly in a hope to find any correlation between them, but in vain as the fluctuation in these is rather insignificant.

In order to conserve the specific name of *violaceus* given by Oka, it is necessary to find out any decisive character, ever described, that can define *B. violaceus* by itself alone. The morphology of larva can be this character. Recently, the colonies of "*Botrylloides violaceus*" collected by Oka and still deposited at the University of Tsukuba were re-examined by Mr. Nishikawa and it was found that one of them harbored the larva with 8 ampullae. At the same time, however, it was made clear that a few colonies from Asamushi in the boreal waters were included in the collection. As the designation of types was not made by Oka, nothing was noted by him on the larva, and no comments were made by him on the distribution either, all these can not combine the larva with 8 ampullae with *violaceus,* but only indicate that *violaceus* was established on the material which was not monospecific but included two species at least. On the other hand, the figure of a botryllid larva given by Hirai (1951) evidently resembles very closely the larva of *B. violaceus,* but there is no comment to indicate the relation between it and *violaceus.* A larva of the same type was given by Tokioka (1953) for the first time in combination with the name of *violaceus.* Very unfortunately, this was overlooked by the present authors and the colonies releasing larvae with 8 ampullae were treated with mistakenly under the name of *violaceus* in many research works carried out at the Research Center (Oka and Watanabe 1959; Mukai and Watanabe 1974, 1975; Mukai 1977; Mukai, Sugimoto and Taneda 1978). The name of *B. violaceus* appeared in these papers should be amended to *B. simodensis.* Though there is a little difference between his observation and the present authors', *B. violaceus,* on which Takeuchi (1980) observed the oozooid



formation, may be regarded as the same as the species here redescribed. According to Tokioka (1951, p. 11), in zooids from the colonies of *B. violaceus* from Akkeshi Bay, the stomach is provided with 12 (rarely 13) plications on the surface. Clearly, this number is considerably greater than in the specimens described in the present paper. Possibly this might imply an intraspecific variation in *B. violaceus*. Further, two new subspecies of *B. violaceus* (1967, 1968, 1970) and a new species of *Botrylloides* (1964) have been proposed by Tokioka. However, full explanations of the morphology of their larvae and their life histories are seemingly requested before they are accepted as really valid taxa. At present, they may be taxonomically of a provisional significance. Noting the similarity in the morphology of zooids, Tokioka (1967, p. 160) was seemingly inclined to consider the unification of *B. leachi* (Savigny), *B. chevalense* Herdman, *B. diegensis* Ritter and Forsyth, and *B. violaceus* into a single taxon represented by the first species. However, this can not be the case, as the larva of *B. leachi* is furnished with 8 ampullae.

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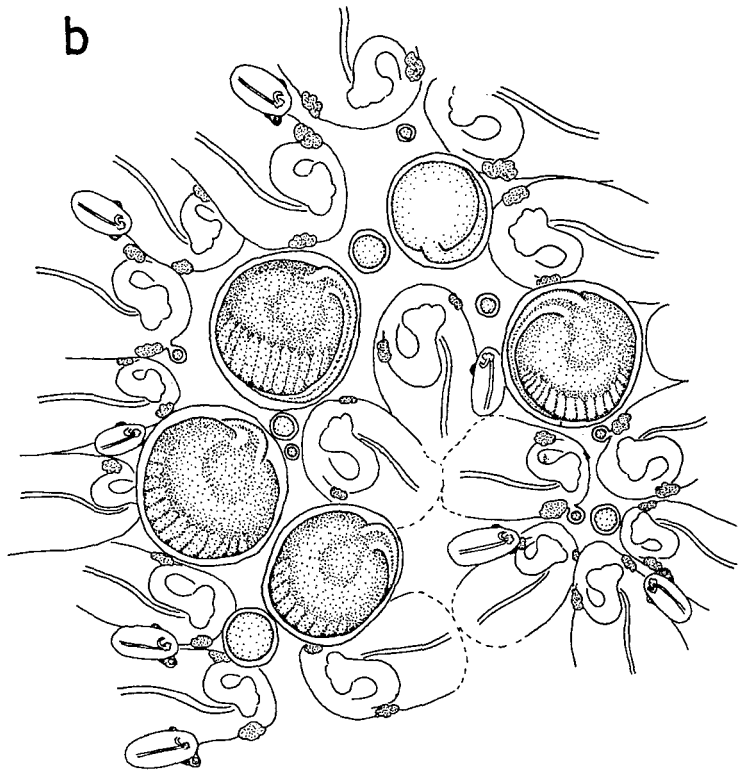

Fig. 5. Colonies in the sexual breeding season, from ventral side. a, *Botrylloides simodensis* n. sp.. All embryos are in the same developmental stage. b, *Botrylloides violaceus* Oka. Embryos are in several different stages.

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